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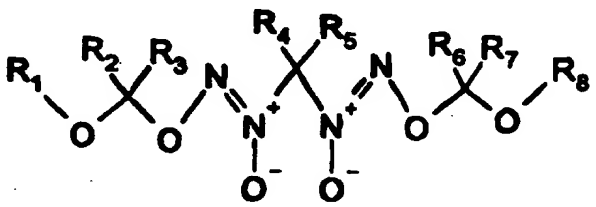
(54) Title: SUBSTITUTED 1,3,9,11-TETRAOXA-4,5,7,8-TETRAAZAUNDECADIENE-4,7-DIOXIDES-5,7, THEIR PREPARATION AND APPLICATION

(57) Abstract

The present invention relates to new chemical compounds - substituted 1,3,9,11-tetraoxa-4,5,7,8-tetraazaundecadiene-4,7-dioxides-5,7 with general structure F1, to methods of their preparation, and to pharmaceutical compositions, containing these compounds. Specifically, such compounds can act as potential donors of nitric oxide NO in living organisms, and as strong cytostatic agents they can be employed in cancer chemotherapy.



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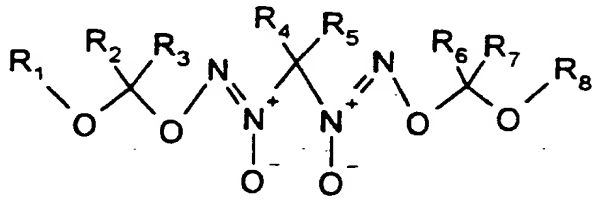
Substituted 1,3,9,11-tetraoxa-4,5,7,8-tetraazaundecadiene-4,7-dioxides-5,7 , their preparation and application

Field of the invention

The present invention relates to new chemical compounds - substituted 1,3,9,11-tetraoxa-4,5,7,8-tetraazaundecadiene-4,7-dioxides-5,7 with general structure F1, to methods of their preparation, and to pharmaceutical compositions, containing these compounds.



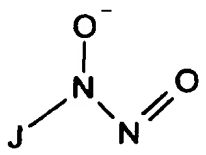
F1

Specifically, such compounds can act as potential donors of nitric oxide NO in living organisms, and as strong cytostatic agents they can be employed in cancer chemotherapy.

Background of the invention

Malignant tumors still remain between the leading human death factors in most of the industrial countries. Therefore elaboration of new, more effective malignant tumors therapy methods, including new anticancer drugs, is a vital necessity. One of possible methods to solve such problem is employment of chemical compounds, which are capable to liberate in vivo nitric oxide NO - an unique biological mediator, having cytotoxic properties (Hibbs J. B. et. al., Biochem. Biophys. Res. Comm., 1988., vol. 157., nr. 1., pp. 87-94).

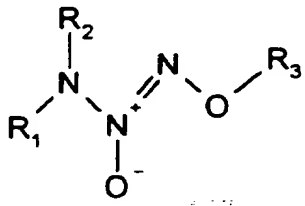
From structural analogues to the claimed compounds F1 in scientific and patent information as nitric oxide NO donors, cytostatic agents and wide spectrum medicines are offered nitric oxide NO and nucleophile adducts of the general structure F2. Here J is a radical, connected with an active moiety via heteroatom, but M is metal, etc. cation (Maragos C. M. et. al., WO 93/20806, publ. 28.10.1993.).



F2

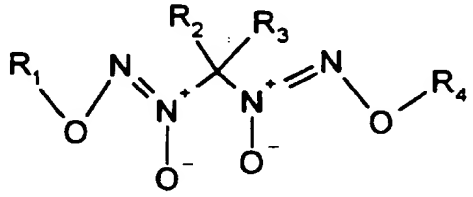
Chemically they represent nitric oxide NO adducts with primary and secondary amines, etc.

Second group of known related compounds are by F2 alkylation obtained substituted alkoxydialkyltriazene-N-oxides with general structure F3, where R_1 - R_3 are different organic residues (Keefer L. K. et. al., WO 93/07114, publ. 15.04.1993.).



F3

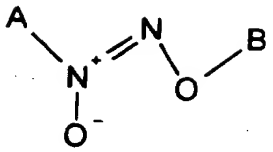
Compounds with similar structure, substituted 1,7-dioxa-2,3,5,6-tetraazaheptadiene-2,5-dioxides-3,5 (see F4) are known as chemical substances (Яндовский В. Н. и др., Ж. Орг. Хим., 1980., т. 16.,вып. 5., стр. 933-936.),



F4

but we cannot found any biomedical information about them. Published analogues (see F4) have R_1=R_4 and they are lower alkyls, any further transformations were done at the central carbon atom.

Compounds with structure F5 are offered (Tsien R. Y. et. al., WO 94/27957) as nitric oxide NO donors after photochemical activation.



F5

In this structure A represents oxygen or nitrogen containing substituent, but B is photolytically unstable group.

Compounds with general structures F2 and F3 have some serious disadvantages:

1. During F2 and F3 decomposition in solutions strongly carcinogenic N-nitrosamines are formed as by-products; this fact is confirmed in literature (Saavedra J. E. et. al., J. Org. Chem., 1992., vol.57., nr.23., pp. 6134-6138.).

2. Compounds F2 and F3 stability is not sufficient enough for galenic drugs preparation. Published half-lifes of adducts F2 in buffered solution with pH=7,4 and 37°C temp. are few minutes up to half a hour (Keefer L. K. et. al., J. Med. Chem., 1991., vol. 34., nr. 11., pp. 3242-3247.). This fact corresponds to the authors conception about direct nitric oxide NO donors, but seriously burdens application of these compounds and galenic forms preparation for medical purposes.

Any biomedical information, as far as we know, is not published on compounds with general structure F4.

In compounds with general structure F5 substituent B (see F5) must be photolytically unstable, which seriously limits their medical application.

For solving mentioned drawbacks we offer as anticancer drugs and potential nitric oxide NO donors substituted 1,3,9,11-tetraoxa-4,5,7,8-tetraazaundecadiene-4,7-dioxides-5,7 (see F1). In comparison with known F2 and F3 they have the following advantages:

1. Nitrosamine formation during destruction of the compound selfsame is ruled out, because molecules F1 do not contain chains from three interconnected nitrogen atoms.

2. Half-life of compounds F1 in aqueous buffer with pH=7,4 and 37°C temp. exceeds 24 hours, which is sufficient for infusion.

3. In comparison with structures F2 and F3, compounds with general structure F1 have additional modification possibilities. By suitable radicals R_1 - R_8 (see F1) selection the whole molecule may be constructed asymmetric at central carbon atom. This gives additional chances for biological activity variation.

Compounds F2 show cytostatic activity (WO 93/20806); they inhibit ^{3}H-thymidine incorporation in DNA of human melanoma A-375-C6 cells to 50% at concentrations 24 - 280 μM.

In our structure-activity relation investigations of diazene oxides compounds with general structure F1 showed high cytostatic activity. Thus, compound with R_1=R_8=methyl, R_5=ethyl, R_2=R_3=R_4=R_6=R_7=H (see F1) at concentration 4 μM shows 27% inhibition of ^{3}H-thymidine incorporation in DNA of mouse P-388 cells. Standart compound N-methyl-N-nitrosourea does not showed ^{3}H-thymidine incorporation inhibition on this model at conc. 1 μM.

Obtained results together with mentioned compounds F1 advantages describes them as promising anticancer pharmaceuticals.

Physico-chemical properties (see experimental part) of claimed compounds with general structure F1 allow their various application for galenic form preparation. Galenic forms for oral use include:

1. Solutions, comprising compounds F1 in therapeutically active amounts in suitable solvents, as water, ethanol, ethanol - water mixtures, dimethylsulfoxide, oils, etc., which in turn may contain other pharmaceutically acceptable ingredients, as well as conservants and stabilizing agents.

2. Tablets, capsules or powders, which contain compounds F1 in therapeutically active amounts. In these forms employment of various colouring, diluting, conserving, etc. modifying components, as microcrystalline cellulose, starch, saccharose, lactose, mannit, gelatin, silicagel, as well as combination with other pharmaceutically acceptable ingredients is allowed.

3. Aerosols, comprising compounds F1 in therapeutically active amounts, which are sprayed by pulverizator or pressurized propellant, as freon, propane, etc.

Galenic forms for parenteral use include sterile injection solutions of compounds F1 in therapeutically active amounts in water or other solvents, as well as microcrystalline compounds F1 suspensions, which additionally may contain conserving, bacteriostatic, buffering agents, antioxidants, salts, as well as other pharmaceutically acceptable ingredients for galenic drug preparation. This includes also ex tempore prepared injection solutions and suspensions.

Experimental part

Example 1.

1,11-dimethyl-1,3,9,11-tetraoxa-4,5,7,8-tetraazaundecadiene-4,7-dioxide-5,7. (A)

In structure F1 $R_1=R_8=$methyl; $R_2=R_3=R_4=R_5=R_6=R_7=H$.

Saturated sodium ethylate solution in anhydrous ethanol was bubbled through with argon, after which gaseous NO was few hours bubbled through, until quantity of precipitate does not more increases. Obtained precipitate was filtered, washed with anhydrous ethanol and dried.

2,0 g of such obtained dry intermediate was suspended in 50 ml of acetonitrile, 5 ml methoxymethylchloride added and resulting suspension was stirred for 6 hr at room temp. After filtration solution was vacuum-evaporated. Remaining oil crystallizes. Product was few times recrystallized from methylethylketone-heptane 1/1.

Colourless crystals 0,25 g.

PMR ($CDCl_3$-TMS): 3,49 (s, 6H, CH_3-O)
5,36 (s, 4H, O-CH_2-O)
5,89 (s, 2H, N-CH_2-N)

Elem. analysis	$C_5H_{12}N_4O_6$	M = 224,175	
calc. %	N 24,99	C 26,79	H 5,40
found	N 24,73	C 26,91	H 5,41

Example 2.

1,11-dimethyl-6-ethyl-1,3,9,11-tetraoxa-4,5,7,8-tetraazaundecadiene-4,7-dioxide-5,7. (B)

In structure F1 R_1=R_8=methyl; R_5=ethyl; R_2=R_3=R_4=R_6=R_7=H.

Saturated sodium n-butylate solution in n-butanol was bubbled through with argon, after which gaseous NO was few hours bubbled through, until quantity of precipitate does not more increases. Obtained precipitate was filtered off, washed with n-butanol and dried.

2,0 g of thus obtained dry intermediate was suspended in 50 ml of acetonitrile, 5 ml methoxymethylchloride added and resulting suspension was stirred for 6 hr at room temp. After filtration solution was vacuum-evaporated. Remaining oil does not crystallizes, therefore few ml of heptane-chloroform 1:1 was added and cooled at -18°C. Precipitated crystals on the next day were filtered off. 0,2 g.

Final purification was done by crystallization from anhydrous ethanol. Colourless crystals, m. p. 41°C.

PMR ($CDCl_3$-TMS) 1,07 (t, 3H, CH_3-C)
2,51 (m, 2H, C-CH_2-C)
3,51 (s, 6H, CH_3-O-)
5,35 (s, 4H, O-CH_2-O)
5,93(t, 1H, central -CH-)

Elem. analysis	$C_7H_{16}N_4O_6$	M = 252,229	
calc. %	N 22,21	C 33,33	H 6,39
found	N 21,95	C 33,34	H 6,35

Example 3. Cytostatic activity.

Mouse lympholeucosis P-388 (mouse line DBA/2) cell suspension was made in RPMI-1640 medium with 5% serum, cell concentration 10^5 cells/ml. Incubation with compounds 2 hr, incubation with ^{3}H-thymidine (1μCi/ml medium) - 1 hr.

	conc (M)	inhibition %
A	$4,5 \times 10^{-4}$	89
	$4,5 \times 10^{-6}$	0
B	4×10^{-4}	48
	4×10^{-6}	27
NMU	10^{-4}	35
	10^{-6}	0

NMU = N-methyl-N-nitrosourea

Substituted 1,3,9,11-tetraoxa-4,5,7,8-tetraazaundecadiene-4,7-dioxides-5,7 , their preparation and application

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1. Hibbs J. B. et. al., Biochem. Biophys. Res. Comm., 1988., vol. 157., nr. 1., pp. 87-94.
2. Maragos C. M. et. al., WO 93/20806, publ. 28.10.1993.
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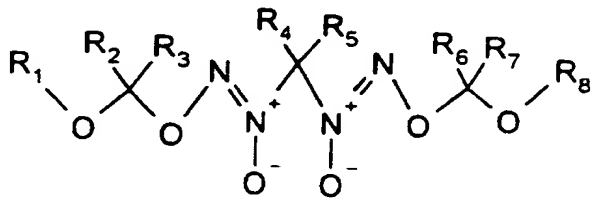
5. Tsien R. Y. et. al., WO 94/27957
6. Saavedra J. E. et. al., J. Org. Chem., 1992., vol.57., nr.23., pp. 6134-6138.
7. Keefer L. K. et. al., J. Med. Chem., 1991., vol. 34., nr. 11., pp. 3242-3247.

Substituted 1,3,9,11-tetraoxa-4,5,7,8-tetraazaundecadiene-4,7-dioxides-5,7 , their preparation and application

We claim:

1. Chemical compounds, substituted 1,3,9,11-tetraoxa-4,5,7,8-tetraazaundecadiene-4,7-dioxides-5,7 of the general structure F1:



F1

2. Pharmaceutical compositions for human and veterinary medicine, which contain in therapeutically active amount specified in claim 1 compounds.

3. Compounds, specified in claim 1, where groups R_1 - R_8 are H or lower alkyls C1 - C4.

4. Pharmaceutical compositions for human and veterinary medicine, which contain in therapeutically active amount specified in claim 3 compounds.

5. Compounds, specified in claim 3, where groups R_1 and R_8 are methyl, groups R_2, R_3, R_6 and R_7 are H, but groups R_4 and R_5 are H or lower alkyls C1 - C4.

6. Pharmaceutical compositions for human and veterinary medicine, which contain in therapeutically active amount specified in claim 5 compounds.

7. Compounds, specified in claim 5, where group R_4=H, but group R_5 is H or ethyl.

8. Pharmaceutical compositions for human and veterinary medicine, which contain in therapeutically active amount specified in claim 7 compounds.

INTERNATIONAL SEARCH REPORT

Inte nal Application No
PCT/LV 96/00001

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C291/08 A61K31/655

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,94 27957 (UNIV CALIFORNIA) 8 December 1994 cited in the application see claims	1-8
A	WO,A,93 20806 (US GOVERNMENT) 28 October 1993 cited in the application see claims	1-8

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEMICAL ABSTRACTS, vol. 093, no. 17, 27 October 1980 Columbus, Ohio, US; abstract no. 167536, YANDOVSKII V N ET AL: "Azo- and azoxy compounds. V. Alkylation of bis(nitrosohydroxylamino)methane salts. Synthesis of 1,7-dialkyl-1,7-dioxa-2,3,5,6-tetraaza-2,5 -heptadiene 3,5-dioxides" XP002005358 see abstract & ZH. ORG. KHIM. (ZORKAE,05147492);80; VOL.16 (5); PP.933-6, LENINGR. GOS. UNIV.;LENINGRAD; USSR, cited in the application	1-8
A	TETRAHEDRON LETT. (TELEAY);69; (32); PP.2689-92, 1969, HARVARD UNIV.;CAMBRIDGE; MASS., XP002005357 WOODWARD R B ET AL: "Methoxazonyl group" see the whole document	1-8
A	CHEMICAL ABSTRACTS, vol. 104, no. 25, 23 June 1986 Columbus, Ohio, US; abstract no. 224533, MARCHENKO G A ET AL: "Derivatives of N'-alkoxydiazene N-oxides. II. Reaction of 2,2-bis(2-methoxy-1-oxodiazenyl)ethanol esters with nucleophiles" XP002005359 see abstract & ZH. ORG. KHIM. (ZORKAE,05147492);85; VOL.21 (7); PP.1429-31, USSR (SU),	1-8
A	CHEMICAL ABSTRACTS, vol. 113, no. 11, 10 September 1990 Columbus, Ohio, US; abstract no. 096995, MARCHENKO G A ET AL: "N'-alkoxydiazene N-oxides. VI. Ethers of N'-alkoxydiazene N-oxides" XP002005651 see abstract & ZH. ORG. KHIM. (ZORKAE,05147492);90; VOL.26 (2); PP.276-9, USSR (SU),	1-8

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		AU-B-	7045194	20-12-94
		EP-A-	0700378	13-03-96
WO-A-9320806	28-10-93	AU-B-	3969793	18-11-93